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Research

Analytical method development and validation for the simultaneous estimation of azelnidipine and telmisartan by RP-HPLC In bulk and tablet dosage forms.

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Abstract

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Azelnidipine and Telmisartan in its pure form as well as in combined marketed formulation. Chromatography was carried out on a Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size column using a mixture of Methanol: Phosphate Buffer (pH-4.2) (37:63% v/v) as the mobile phase at a flow rate of 1.0ml/min, thedetection was carried out at 260 nm. The retention time of the Azelnidipine and Telmisartan was found to be was 2.133, 3.692±0.02 min respectively. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Azelnidipine and 10-30mg/ml of Telmisartan.The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Azelnidipine and Telmisartan, RP-HPLC, Validation, Accuracy, Precision.

INTRODUCTION

Chromatography

The chromatographywas discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma , and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.

“] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. "Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).

High Performance Liquid Chromatography (HPLC) [6]

The acronym *HPLC*, coined by the Late Prof. Csaba Horvath for his 1970 Pittconpaper, originally indicated the fact that high pressure was used to generate the flow required for liquid chromatography in packed columns. In the beginning, pumps only had a pressure capability of 500 psi [35 bars]. This was called *high pressure liquid chromatography*, or HPLC. The early 1970s saw a tremendous leap in technology. These new HPLC instruments could develop up to 6,000 psi [400 bars] of pressure, and incorporated improved injectors, detectors, and columns. With continued advances in performance during this time [smaller particles, even higher pressure], the acronym HPLC remained the same, but the name was changed to high performance liquid chromatography. High Performance Liquid Chromatography is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantitative the compounds that are present in any sample that can be dissolved in a liquid. Today, compounds in trace concentrations as low as *parts per trillion* (ppt) may easily be identified. HPLC can be, and has been, applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental matrices, forensic samples, and industrial chemicals.

Normal phase chromatography

Normal phase HPLC (NP-HPLC) was the first kind of HPLC chemistry used, and separate analytes, based on polarity. This method uses a polar stationary phase and a non-polar mobile phase, and is used when the analyte is fairly polar in nature. The polar analyte associates with and is retained by the polar stationary phase. Absorption strengths increase with increase in analyte polarity, and the interaction between the polar analyte and the polar stationary phase increases the elution time. The interaction strength not only depends on the functional groups in the analyte molecule, but also on stearic factors and structural isomers is often resolved from one another. Use of more polar solvents in the mobile phase will decrease the retention time of the analyte while more hydrophobic solvents tend to increase retention times. Particularly polar solvents in a mixture tend to deactivate the column by occupying the stationary phase surface.

Reversed phase chromatography (RPC)

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention Time (R_t) is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent. The pharmaceutical industry regularly employs RPC to qualify drugs before their release.

RPC operates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar analyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. The energy released in this process is proportional to the surface tension of the eluent (water: 73 erg/cm^2, methanol: 22 erg/cm^2) and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding less-polar solvent (MeOH, ACN) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically changing the polarity of the mobile phase during the course of the analysis.

Isocratic flow and gradient elution

A separation in which the mobile phase composition remains constant throughout the procedure is termed isocratic (meaning constant composition). The word was coined by Csaba Horvath, who was one of the pioneers of HPLC. The mobile phase composition does not have to remain constant. A separation in which the mobile phase composition is changed during the separation process is described as a gradient elution. One example is a gradient starting at 10% methanol and ending at 90% methanol after 20 minutes. The two components of the mobile phase are typically termed "A" and "B"; A is the "weak" solvent which allows the solute to elute only slowly, while B is the "strong" solvent which rapidly elutes the solutes from the column. In reverse-phase chromatography, solvent A is often water or an aqueous buffer, while B is an organic solvent miscible with water, such as Acetonitrile, methanol, THF, or isopropanol.

Working Principle of HPLC [8]

The components of a basic High-Performance Liquid Chromatography [HPLC] system are shown in the simple diagram in figure 5. A reservoir holds the solvent [called the mobile phase, because it moves]. A high-pressure pump [solvent delivery system or solvent manager] is used to generate and meter a specified flow rate of mobile phase, typically millilitres per minute. An injector is able to introduce [inject] the sample into the continuously flowing mobile phase stream that carries the sample into the HPLC column.

The column contains the chromatographic packing material needed to effect the separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to seethe separated compound bands as they elute from the HPLC column. The mobile phase exits the detector and can be sent to waste, or collected, as desired. When the mobile phase contains a separated compound band, HPLC provides the ability to collect this fraction of the elute containing that purified compound for further study. This is called preparative chromatography.

The detector is wired to the computer data station, the HPLC system component that records the electrical signal needed to generate the chromatogram on its display and to identify and quantitative the concentration of the sample constituents. Since sample compound characteristics can be very different, several types of detectors have been developed. For example, if a compound can absorb Ultra Violet light, a UV-absorbance detector is used. If the compound does not have either of these characteristics, a more universal type of detector is used, such as an Evaporative-Light-Scattering Detector [ELSD]. The most powerful approach is the use multiple detectors in series. For example, a UV and/or ELSD detector may be used in combination with a Mass Spectrometer [MS] to analyze the results of the chromatographic separation. This provides, from a single injection, more comprehensive information about an analyte. The practice of coupling a mass spectrometer to an HPLC system is called LC/MS.

MATERIALS AND METHODS

Azelnidipine,Telmisartan-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Potassium Dihydrogen Phosphate-Finar Chemicals.

HPLC method development
Trails

Preparation of standard solution: Accurately weigh and transfer 10 mg of Azelnidipine and Telmisartan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.4ml of Azelnidipine and 0.2ml of Telmisartan from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was methanol: Water, Methanol: Phosphate buffer and ACN: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v) in proportion 37:63 v/v respectively.

Optimization of Column: The method was performed with various C18columns like Symmetry, X terra and ODS column. Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used	:	Waters Alliance 2695 HPLC with PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Mobile phase	:	Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Flow rate	:	1ml/min
Wavelength	:	260 nm
Injection volume	:	10µl
Run time	:	6minutes

Method validation

Preparation of buffer and mobile phase

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.2): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.2 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of Mobile Phase: Accurately measured 350 ml (35%) of TEA buffer and 650 ml of HPLC Methanol (65%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio	: Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v)
Column	: Phenomenex Luna C18 (4.6mm×250mm) 5µm particle size
Column temperature	: 35ºC
Wavelength	: 260 nm
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 6minutes

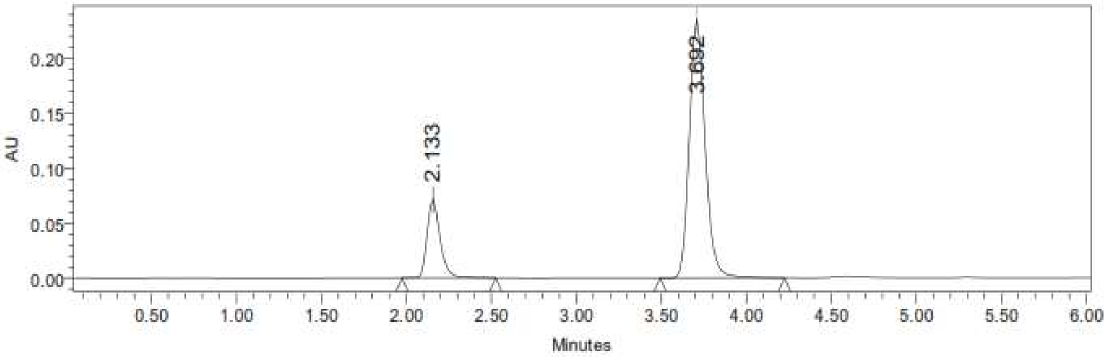

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Azelnidipine	2.133	526389	86756	1.56	5679	
2	Telmisartan	3.692	1687285	367532	1.79	8685	9.8

From the above chromatogram it was observed that the Azelnidipine and Telmisartan peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram

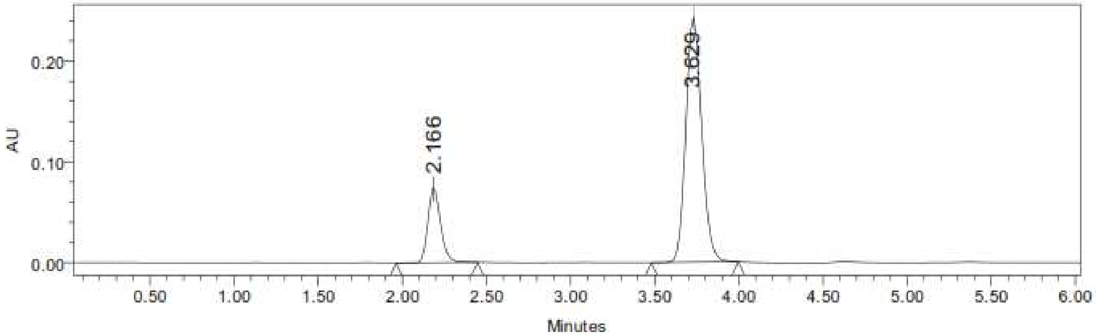

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No.	Name	Rt	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Azelnidipine	2.166	536587	77464	1.57	5789	
2	Telmisartan	3.629	1695846	378564	1.80	8795	10.01

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Azelnidipine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Azelnidipine	2.152	526358	86598	5695	1.56
2	Azelnidipine	2.157	526548	86254	5652	1.57
3	Azelnidipine	2.141	526854	86598	5627	1.56
4	Azelnidipine	2.133	526598	86245	5692	1.57
5	Azelnidipine	2.166	524874	86521	5641	1.56
Mean			526246.4			
Std. Dev.			787.353			
% RSD			0.149617			

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Telmisartan

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Resolution
1	Telmisartan	3.674	1682821	1686958	8659	1.56	9.8
2	Telmisartan	3.631	1682726	1685745	8675	1.57	9.9
3	Telmisartan	3.625	1687361	1685421	8692	1.56	9.8
4	Telmisartan	3.692	1682811	1685242	8642	1.57	9.8
5	Telmisartan	3.629	1683816	1685364	8635	1.58	9.8
Mean			1683907				
Std. Dev.			1982.03				
% RSD			0.117704				

- *%RSD of five different sample solutions should not more than 2.*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Assay (Standard)

Table 5: Peak results for assay standard of Azelnidipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Azelnidipine	2.152	526358	86598	1.56	5698	1
2	Azelnidipine	2.198	526584	86784	1.57	5687	2
3	Azelnidipine	2.179	529658	86253	1.56	5639	3

Table 6: Peak results for assay standard of Telmisartan

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Telmisartan	3.646	1687589	365879	1.80	8659	1
2	Telmisartan	3.604	1685987	365854	1.79	8697	2
3	Telmisartan	3.610	1685974	369854	1.80	8675	3

Assay (Sample)

Table 7: Peak results for Assay sample of Azelnidipine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Azelnidipine	2.152	536859	87584	1.58	5789	1

2	Azelnidipine	2.150	532654	87965	1.59	5784	2
3	Azelnidipine	2.187	532685	87465	1.58	5769	3

Table 8: Peak results for Assay sample of Telmisartan

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Telmisartan	3.646	1698568	378562	1.81	8759	1
2	Telmisartan	3.651	1698574	375847	1.80	8795	2
3	Telmisartan	3.601	1698547	376584	1.81	8745	3

%ASSAY =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

= 99.89%

The % purity of Azelnidipine and Telmisartan in pharmaceutical dosage form was found to be 99.89%

Linearity

Chromatographic data for linearity study of azelnidipine

Table 9: Chromatographic Data for Linearity Study of Azelnidipine

Concentration µg/ml	Average Peak Area
20	272897
30	402986
40	526389
50	649785
60	769287

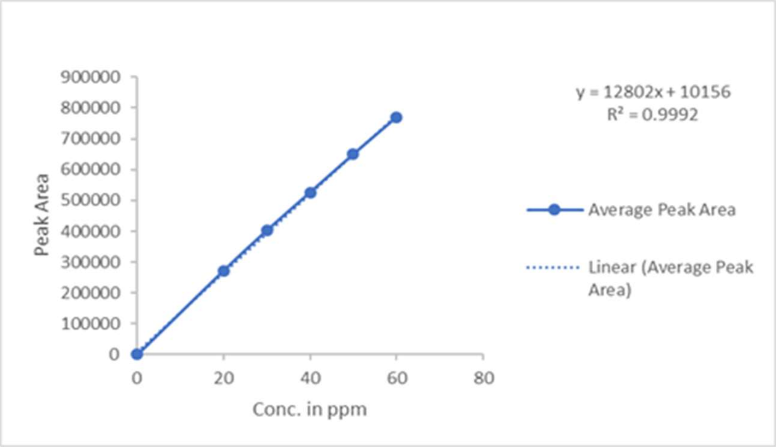

Fig 3: Calibration Curve of Azelnidipine

Chromatographic data for linearity study of telmisartan

Table 10: Chromatographic Data for Linearity Study of Telmisartan

Concentration µg/ml	Average Peak Area
10	1000237
15	1448768
20	1887285
25	2365897
30	2826845

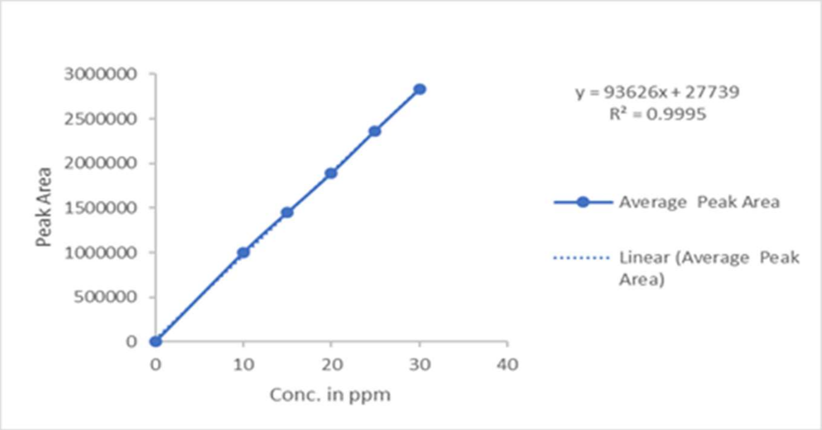

Fig 4: Calibration Curve of Telmisartan

Precision
Repeatability

Table 11: Results of repeatability for Azelnidipine

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Azelnidipine	2.157	526358	86598	5689	1.56
2	Azelnidipine	2.159	524856	86542	5687	1.57
3	Azelnidipine	2.186	526985	86578	5684	1.56
4	Azelnidipine	2.160	528654	86354	5689	1.56
5	Azelnidipine	2.170	528457	86958	5639	1.56
Mean			527062			
Std.dev			1569.114			
%RSD			0.297709			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 12: Results of Repeatability for Telmisartan

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	3.603	1687589	367859	8659	1.79
2	Telmisartan	3.608	1685987	368547	8679	1.80
3	Telmisartan	3.600	1685987	367985	8645	1.80
4	Telmisartan	3.696	1685754	365874	8695	1.79
5	Telmisartan	3.629	1685985	364589	8625	1.79
Mean			1686260			
Std.Dev			749.493			
%RSD			0.044447			

Intermediate precision
Day 1

Table 13: Results of Intermediate precision for Azelnidipine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	%Assay
1	Azelnidipine	2.198	546585	87589	5898	1.58	100%
2	Azelnidipine	2.196	548758	87985	5879	1.59	100%
3	Azelnidipine	2.160	549854	87452	5868	1.58	100%
4	Azelnidipine	2.160	548798	87421	5847	1.59	100%

5	Azelnidipine	2.160	542659	87963	5896	1.58	100%
6	Azelnidipine	2.186	548754	87254	5874	1.59	100%
Mean			547568				
Std. Dev.			2631.576				
% RSD			0.480593				

- *%RSD of five different sample solutions should not more than 2*

Table 14: Results of Intermediate precision for Telmisartan

S.No.	Peak Name	Rt	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution	%Assay
1	Telmisartan	3.623	1698587	385482	8789	1.81	9.8	98%
2	Telmisartan	3.611	1698574	385698	8759	1.80	9.8	98.2%
3	Telmisartan	3.696	1698532	385748	8754	1.81	9.9	98.7%
4	Telmisartan	3.696	1698574	386958	8754	1.81	10.01	99.7%
5	Telmisartan	3.696	1698532	385755	5798	1.80	9.98	98.5%
6	Telmisartan	3.642	1698547	386558	8762	1.80	10.02	98.2%
Mean			1698558					
Std. Dev.			23.77113					
% RSD			0.001399					

- *%RSD of five different sample solutions should not more than 2*

Day 2

Table 15: Results of Intermediate precision Day 2 for Azelnidipine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Azelnidipine	2.198	536854	8758	5789	1.58
2	Azelnidipine	2.196	536985	8795	5726	1.59
3	Azelnidipine	2.178	536587	8746	5742	1.58
4	Azelnidipine	2.142	532546	8754	5746	1.59
5	Azelnidipine	2.177	534587	8725	5798	1.58
6	Azelnidipine	2.177	538598	8726	5785	1.59
Mean			536026.2			
Std. Dev.			2131.492			
% RSD			0.397647			

- *%RSD of five different sample solutions should not more than 2.*

Table 16: Results of Intermediate precision Day 2 for Telmisartan

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing	Resolution
1	Telmisartan	3.611	1678598	356875	8875	1.82	9.9
2	Telmisartan	3.623	1678985	358985	8856	1.83	10.01
3	Telmisartan	3.684	1678984	358754	8862	1.82	9.9
4	Telmisartan	3.697	1678985	352412	8849	1.83	10.01
5	Telmisartan	3.684	1678549	358987	8873	1.82	9.9
6	Telmisartan	3.684	1678984	358986	8842	1.83	10.01
Mean			1678848				
Std. Dev.			212.8048				
% RSD			0.012676				

- *%RSD of five different sample solutions should not more than 2.*

Accuracy

Table 17: The accuracy results for Azelnidipine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	267011.3	20	20.063	100.315%	100.28%
100%	523752.3	40	40.118	100.295%	
150%	778457.3	60	60.133	100.221%	

- *The percentage recovery was found to be within the limit (98-102%).*

Table 18: The accuracy results for Telmisartan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972876.3	10	10.094	100.94%	100.48%
100%	1900122	20	19.998	99.99%	
150%	2851152	30	30.156	100.52%	

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 19: Results for robustness

Azelnidipine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526389	2.133	5679	1.56
Less Flow rate of 0.9 mL/min	542685	2.210	5264	1.54
More Flow rate of 1.1 mL/min	526483	2.184	5426	1.52
Less organic phase	516854	2.200	5163	1.57
More Organic phase	506898	2.172	5098	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Telmisartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1687285	3.692	8685	1.79
Less Flow rate of 0.9 mL/min	1725468	4.498	8265	1.68
More Flow rate of 1.1 mL/min	1652847	3.505	8415	1.59
Less organic phase	1687485	4.504	8326	1.62
More organic phase	1674524	3.512	8415	1.63

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Azelnidipine and Telmisartan in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Azelnidipine was found to be freely soluble in chloroform, soluble in water and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. Telmisartan was found to be soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide, soluble in water. Methanol: Phosphate Buffer (pH-4.2) (37:63 v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Azelnidipine and Telmisartanin bulk drug and in Pharmaceutical dosage forms.

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